Attorney Docket No. 5218-39B PATENT

In re: Anagnostou et al. Confirmation No. 9917
Serial No.: 09/525,797 Group Art Unit: 1642
Filed: March 15, 2000 Examiner: Karen A. Canella
For: *METHOD OF TREATING ENDOTHELIAL INJURY*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

May 13, 2008

Commissioner for Patents
Post Office Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R § 1.132
OF GEORGE SIGOUNAS, Ph.D.

Sir/Madam:

I, George Sigounas, Ph.D., do hereby declare and say as follows:

1. I received my Ph.D. from Boston University in Cellular Biology. I am currently Professor of Medicine at East Carolina University School of Medicine in Greenville, North Carolina. I am a co-inventor on the above-identified patent application.

2. I have reviewed the Office Action dated November 16, 2007 issued in connection with the above-identified patent application.

3. I have conducted and/or reviewed studies presented in the above-identified patent application and those presented in the Sigounas Declarations referenced in the Office Action dated November 16, 2007.

4. The erythropoietin (EPO) concentration of 60 U/kg mentioned in the referenced Sigounas Declarations is a typographical error. The accurate dosage employed was 60 units of EPO per mouse. In our *in vivo* experiments, tumor-bearing mice were injected with 40-60 units of EPO per mouse. When the mice were received by the East

Carolina University animal care and use facility, their weight was approximately 15-20 grams and their age was approximately 6-8 weeks. For adaptation purposes, the animals were kept in the animal facility for at least two weeks generally prior to experimentation. During this period, most of the animals gained weight. Thus, a 30 gram mouse injected with 60 units of EPO received approximately 2000 U/kg EPO.

In our *in vitro* studies, 5-20 U/mL of EPO, combined with the chemotherapeutic drugs, induced the highest growth inhibition of endothelial cells. Based on these observations, a 50 mL culture generally requires 250-1000 units of EPO. The blood of each individual or animal is approximately 5% of the total body weight. For example, a 20 g mouse has approximately 1 mL of blood, and thus, 1 kg of mouse would correspond to approximately 50 mL of blood. Thus, based on *in vitro* results, EPO concentrations higher than 250 U/kg combined with chemotherapy would inhibit the growth of endothelial cells. Therefore, 750-2000 U/kg of EPO is within the expected effective range.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

George Sigounas

George Sigounas, Ph.D.

5-14-08

Date